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Research Article

Dead or alive: the effect of shells and living individuals of *Sinanodonta woodiana* (Lea, 1834) on habitat selection and behaviour of European unionid bivalves

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Abstract

1. Ecosystem engineering freshwater bivalves, burrowing in the substratum and accumulating shell deposits, transform bottom habitats. Especially the invasive Asian bivalve *Sinanodonta woodiana* (SW), due to its rapid growth, large size, and high fecundity, can affect benthic communities. Here, we determined its effect on habitat selection and behaviour of endangered native bivalves, *Anodonta cygnea* and *Unio tumidus*.

2. We conducted laboratory preference assays (Experiment 1: choice between two substrata) exposing the native bivalves to pure sand (control), shells (several densities on the sand surface or burrowed), or living SW. Then, we tested their locomotion and burrowing (Experiment 2) on pure sand and substrata contaminated with shells or living SW.

3. In Experiment 1, native bivalves avoided shells, but not living SW. Burrowed and larger shells were avoided compared with those on the surface and smaller ones, respectively.

4. In Experiment 2, *U. tumidus* exposed to SW delayed activity initiation (in response to living bivalves), increased locomotion (living bivalves, surface shells), and reduced burrowing depth (living bivalves, all shells). *Anodonta cygnea* exposed to SW reduced locomotion speed (living bivalves, shells), and reduced burrowing duration (burrowed shells) and depth (living bivalves, burrowed shells).

5. SW (especially shell beds) constitutes another emerging threat to native bivalves, impairing their burrowing and inducting active avoidance. As SW expands its distribution with climate warming, the range and strength of its impact is likely to increase, reducing the area available to native bivalves, exposing them to environmental dangers (due to burrowing limitation) and deteriorating physical condition (energetic resources used for excessive locomotion).

Key words: Behaviour, biological invasions, Bivalvia, ecosystem engineers, habitat selection, interspecific interactions, species displacement, unionid mussels

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Introduction

Bivalves of the Unionidae family are freshwater bottom dwellers of limited mobility (Curley et al. 2021). Through filter-feeding, bivalves can considerably modulate the availability of resources for other organisms by transferring suspended particles to the bottom sediments (Boeker et al. 2016; Pouil et al. 2021). Both living bivalves and their empty shells, accumulating in the environment long after the animal death, constitute unique hard structures affecting community functioning (Gutiérrez et al. 2003). Therefore, bivalves are considered to be ecosystem engineers and their extinction, overpopulation or changes in their taxonomic composition lead to habitat modifications with cascading effects on the aquatic community and ecosystem services provided by these animals (Vaughn 2018).

Freshwater bivalves are threatened globally by human impact, including climate change and pressure from non-native species (Strayer 2008, Blackburn et al. 2014; Bacher et al. 2018). The number of alien bivalve species and sizes of their populations have been growing for several decades (Seebens et al. 2021; Latombe et al. 2022). Accordingly, the pressure from non-native bivalves is considered a major threat to their native counterparts (Mack et al. 2000; Pyšek et al. 2010), especially due to their ecosystem engineering properties and resulting habitat transformations (Bódis et al. 2014a; Douda et al. 2024).

The Chinese pond mussels of the genus *Sinanodonta* are unionid bivalves native to Eastern Asia, but invasive in other parts of the world. Recent genetic studies have shown that invasive lineages belong to three species: (i) *S. woodiana* (Lea, 1834), the "temperate invasive" lineage, native to southern China and invasive in Europe, as well as in western and central Asia, observed probably in Africa (finding needs genetic confirmation (Bensaâd-Bendjedid et al. 2023)); (ii) *S. pacifica* (Heude, 1878) the "tropical invasive" lineage, whose native area is Taiwan and eastern China, whereas it is invasive in North America, southern Asia and Iraq, and (iii) *S. lauta* (Martens, 1877), originating from Japan, the Korean Peninsula and eastern Russia, and invading central and southern Asia (Douda et al. 2024). In Europe, only *S. woodiana* ("temperate invasive" lineage) occurs, likely originating from a single introduction event (Konečný et al. 2018). Therefore, we will focus on this species in the current study. In colder regions (such as central and eastern Europe), its spread was initially limited to artificially heated waters (Urbańska et al. 2012), but, over the past two decades, it has accelerated and extended to habitats of natural thermal regime (Bogan et al. 2011; Bolotov et al. 2016; Lopes-Lima et al. 2017; Bespalaya et al. 2018; Kondakov et al. 2018, 2020; Konečný et al. 2018). Substratum preferences of *S. woodiana* overlap with those of the native European Unionidae (Poznańska-Kakareko et al. 2021) indicating a high risk of competitive tensions between them (Douda and Čadková 2018). This invasive bivalve exhibits a number of competitive advantages over the native Unionidae, including the higher rate of host infection by its parasitic glochidium larvae, faster development and growth rate (Douda et al. 2012; Huber and Geist 2019) and higher fecundity (Labecka and Domagala 2018; Labecka and Czarnoleski 2019).

Another potential mechanism of the impact of *S. woodiana* on native Unionidae can be the transformation of the bottom by living individuals and shell beds formed after the bivalve death (Bódis et al. 2014a; Nakano 2023). *Sinanodonta woodiana* is a large (up to 26 cm) and fast-growing species (Urbańska et al. 2019)

reaching high densities. In a Polish lake, densities of 68 ind. m^{-2} and 27.9 kg m^{-2} were observed (Kraszewski and Zdanowski 2007), which is the highest density of this species reported for Europe. In other European countries, the density ranges from a few ind. m^{-2} in Ukraine (Yermoshyna and Pavliuchenko 2021) to c.a. 50 ind. m^{-2} in Hungary and Italy (Benkő-Kiss et al. 2013; Kamburska et al. 2013). Over time, shells accumulate on the bottom surface and in the sediments, outnumbering living individuals and forming a layer significantly changing the substratum quality. More than 280 ind. m^{-2} (counting both valves as one individual) were noted by Bódis et al. (2014a). Shells reduce the near-bottom current velocity, limit the access of light to the bottom, and increase microhabitat heterogeneity (Gutiérrez et al. 2003). Moreover, shells (especially large ones) can create physical barriers limiting bivalve movement and burrowing, and thus degrading the living conditions for these organisms. It is likely that the effect of shell beds formed by *S. woodiana* will be stronger than that of shells originating from the native species present in the environment before the invasion, due to the shorter lifespan of *S. woodiana*. It can live up to a maximum of 12 years (Spyra et al. 2012), compared to the maximum lifespan of 37 and 21 years exhibited by native *Anodonta cygnea* (Linnaeus, 1758) and *Unio tumidus* Philipsson, 1788, respectively (Aldridge 1999). This results in a faster accumulation rate of *S. woodiana* shells on the bottom. Moreover, its shells are larger, thus constituting larger and heavier physical obstacles in the substratum.

Knowledge of the responses of the native bivalves to the presence of *S. woodiana* will help understand the mechanisms and magnitude of its impact, as well as develop methods of dealing with this new threat. The aim of our study was to determine mechanical effects of substratum contamination with living individuals and shells of *S. woodiana* on behaviour (habitat selection, locomotion and burrowing) of two native European unionid bivalves: *A. cygnea* and *U. tumidus*. Their numbers are constantly decreasing worldwide (Lopes-Lima et al. 2017), and they are protected by law in several countries (Van Damme 2011; Lopes-Lima 2014). These species were selected due to their reported coexistence with *S. woodiana* (Lajtner and Crnčan 2011; Beran 2019) and similar habitat preferences (Poznańska-Kakareko et al. 2021). We hypothesized as follows: (1) native bivalves would avoid substrata contaminated with *S. woodiana*. (2) The adverse effect of shell beds on bivalve preferences would result from deteriorated burrowing and/or locomotion. Alternatively, increased locomotion might indicate active avoidance of the substratum contaminated by *S. woodiana*. (3) Empty shell beds would affect native bivalves to a greater extent than living *S. woodiana*. This might be due to (i) variable shell positions in the sediments (horizontally or vertically, on the surface or burrowed) compared to always vertically burrowed living bivalves (see Suppl. material 1: fig. S1), or (ii) the presence of sharp shell edges irritating the foot of moving bivalves. Options (i) or (ii) would be supported by stronger unionid responses to shells present on the sediment surface or burrowed, respectively. (4) The effect of *S. woodiana* shells on native bivalves would differ from that of native shell beds. A presumably stronger effect of the invader (compared to the shells of native bivalves) would result from either interspecific differences in shell structure (resulting in different unionid responses to shells of various species presented at the same sizes and densities) or the larger size of *S. woodiana* shells (resulting in stronger responses of unionids to larger shells).

Materials and methods

Bivalve collection in the field

Anodonta cygnea, *U. tumidus* and *S. woodiana* (shells and living individuals) were collected in early autumn from the sandy/muddy bottom (depth: 1.5–2.5 m) from the Włocławski Reservoir on the River Vistula, Central Poland (52°37'04"N, 19°19'42"E) by scuba divers. This site represents a natural thermal regime for central Europe, and has been recently invaded by *S. woodiana* (Cichy et al. 2016; Douda et al. 2024). Currently, all the study species co-exist at the location and the invader is constantly increasing its abundance and range (personal observation). We obtained *S. woodiana* shells from freshly killed individuals (on the day of collection), while native Unionidae shells were collected as fresh shells (uncorroded, undamaged) lying on the bottom of the reservoir (to avoid killing the native species). The collected bivalves were transported to the laboratory in buckets with substratum and water from the reservoir and tested after two weeks of acclimation.

Stocking conditions

Living bivalves (each species separately) and empty shells were kept in 350-L stock tanks (20–30 individuals per tank) equipped with internal filters and aeration systems, with the bottom covered by a few cm deep layer of sand taken from the collection site. The stock/experimental room was equipped with a photoperiod system (light/dark cycle: 12:12 h) imitating the natural day-night cycle, and air-conditioning which kept the water temperature in the tanks at the level similar to that observed in the reservoir during bivalve collection. We checked the water quality in the stock and experimental tanks using a multimeter Multi340i (WTW GmbH, Weilheim, Germany). The water parameters were within the following ranges: oxygen content: 7.37–7.77 mg ml^{-1} (82.9–87.2%); temperature: 18.4–20.1 °C; pH: 8.01–8.67; conductivity: 643–827 µS cm^{-1}. The bivalves were fed twice a week with a suspension of dried *Chlorella* algae ("*Chlorella* super alga", Meridian company, Poland) in a concentration of 5 mg L^{-1} (Douda and Čadková 2018).

Experiment 1: Habitat selection

Tests were conducted in 30 × 30 × 30 cm tanks divided into halves (Suppl. material 1: figs S1, S2). Each half was filled with a different substratum (see below) to a depth of 10 cm. Then, the tank was filled with conditioned (settled and aerated for at least 48 h) tap water (a 10-cm layer above the substratum surface). One bivalve individual was introduced in the central part of the tank with its ventral side down and anteroposterior axis parallel to the border between the substrata. After 24 h, the location of the tested individual was checked (choosing one of the two substrata). Each configuration of substratum types was repeated 30 times per species. We used the following substratum types:

(1) Sand (grain diameter range: 0.2–1.4 mm; median: 0.63 mm) obtained from the bivalve collection site. This material was earlier found to be preferred by all the species tested (Poznańska-Kakareko et al. 2021). The pure sand was used as a control. The same sand type was contaminated with *S. woodiana* to create other substratum types.

(2) Empty *S. woodiana* shells, small (mean length: 7.6 cm) or large (12.6 cm) (Table 1), composed of two valves connected naturally by the ligamentum. The shells were burrowed under the surface (covered with sand) or laid on the surface of the sandy substratum. We used these two shell position variants because a bivalve usually dies on the surface and, after some time, due to hydrodynamics and sedimentation, its shell becomes burrowed. This is especially visible in the case of mass mortalities, when large quantities of empty shells cover the surface of the bottom (Bódis et al. 2014b). Shell arrangement in/on the substratum (lying on their side or put vertically with their ventral surface down) was random (Suppl. material 1: fig. S1) to reflect their arrangement in the field. Shells were randomly put on the sand within the tank half they were assigned to (Suppl. material 1: fig. S1). Then, those assigned to the burrowed variant were gently and thoroughly covered with sand to fill all the spaces between them.
(3) Living individuals of *S. woodiana* (mean length of 11.6 cm, corresponding to the large shells; Table 1) immobilized by adhesive tape applied to the front of the shell (to prevent their relocation) and burrowed in the sandy bottom at 75% of their length (Suppl. material 1: fig. S4). The immobilized bivalves could not extend their foot and move, which was necessary to keep them in their positions within the assigned half of the tank (Suppl. material 1: fig. S4). However, they could partially open their valves, pull out the siphons, filter water and breathe.

All bivalves and shells were thoroughly rinsed with water before use and biofilm and adhering debris were scrubbed from their surfaces. The sand was rinsed and dried in a laboratory dryer (SLW 115 STD Multiserw-Morek, Poland) at 60 °C for 6 h before use to eliminate any organisms that could potentially affect the results of the experiment. It should be noted that the size defined as large in our study is not of the maximum size of *S. woodiana* (26 cm, Urbańska et al. 2019). These, however, can be generally collected from warmer waters, whereas we used the size range commonly available at the collection site of the thermal regime natural for central Europe.

First, we checked unionid selectivity between the pure sand and various shell densities (small or large, on the surface or burrowed). We started the experiment with a density of 133 ind. m^{-2} (6 shells per tank, two valves counted as one individual), i.e. twice as much as the maximum field density observed in heated waters. Then, we continued with the lower (67 ind. m^{-2}, 3 shells per tank) or higher (200 ind. m^{-2}, 9 shells per tank), depending on the presence or absence of a significant reaction to the initial density, respectively. This allowed us to determine the minimum effective density capable of influencing bivalve behaviour. We also confronted the pure sand with living *S. woodiana* at a density of 133 ind. m^{-2}. We did not use higher densities of living *S. woodiana*, as they would have been unrealistic given the maximum density reported in the wild (Kraszewski and Zdanowski 2007).

Moreover, we confronted the following: (i) burrowed shells vs. shells present on the sediment surface (using small shells at a density of 200 ind. m^{-2}) to check if shell position makes a difference, (ii) living *S. woodiana* vs. large burrowed shells (133 ind. m^{-2}) and (iii) burrowed small vs. burrowed large shells (200 vs. 133 ind. m^{-2}, corresponding to the same total volumes occupied by shells of the two sizes) to check whether bivalves respond differently to shell beds composed of shells of different sizes, (iv) native unionid shells vs. pure sand, (v) native unionid shells vs. small *S. woodiana* shells, to check if unionid responses to shells depend on shell origin.

Table 1. Total length of bivalves and shells [cm].

	Mean	SD	Range
A. cygnea	10.4	0.83	9.0-13.0
U. tumidus	7.2	0.60	6.5-8.5
Native bivalve shells*	7.0	0.88	5.5-9.0
S. woodiana living individuals	11.6	0.84	10.5-13.0
S. woodiana small shells	7.6	1.14	5.5-10.0
S. woodiana large shells	12.6	1.24	10.5-14.5

SD - standard deviation, * - *U. tumidus* with small admixture of *U. pictorum* and *A. anatina*.

Native shell beds were composed mostly of *U. tumidus* shells with a small admixture of *U. pictorum* and *A. anatina* (as they occurred in the field). They were of a size considered in the current study to be small (Table 1) and burrowed in sand (in this form they triggered stronger responses in earlier trials) at a density of 200 ind. m^{-2} (effective density of small shells in earlier trials, see the Results). Treatments (iii)-(v) were conducted using only *U. tumidus*, because both native species responded similarly in earlier trials (see the Results), and we wanted to limit the use of the legally protected and endangered *A. cygnea*.

Furthermore, we tested the habitat preferences of *S. woodiana* for: (i) small burrowed conspecific shells (200 ind. m^{-2}) vs. pure sand and (ii) small burrowed conspecific shells vs. shells of native unionids (200 ind. m^{-2}) to check whether and how this species responds to shell beds. All the pairwise comparisons carried out within Experiment 1 are listed in Suppl. material 1: table S1.

Experiment 2: Bivalve mobility and burrowing

To test the effect of living *S. woodiana* and its empty shells on the locomotion and burrowing of *A. cygnea* and *U. tumidus*, we used tanks (40 × 30 × 35 cm) with a 10-cm layer of sand covered by the conditioned tap water (10 cm above the substratum) (Suppl. material 1: fig. S2). As substrata, we used small *S. woodiana* shells (i) burrowed or (ii) lying on the sand surface, at a density of 200 ind. m^{-2}, as well as (iii) living *S. woodiana* (133 ind. m^{-2}) (Suppl. material 1: table S2). We used *S. woodiana* densities found to be avoided by the native unionids in Experiment 1 (see the Results). In the control treatment, bivalves were tested on (iv) pure sand without shells. A single substratum type was placed in each experimental tank. We introduced a single bivalve to the centre of the tank and recorded its behaviour using a CCTV camera (Samsung SNB-6004, South Korea) for 24 h. The tests were replicated 15 times for each substratum and species. While watching the videos, we determined the following: (i) movement initiation time (time from the bivalve introduction to the first movement), (ii) locomotion duration, (iii) locomotion distance, (iv) locomotion speed (excluding periods of immobility), (v) duration of burrowing activity, (vi) mean burrowing level [%]. Every minute, we estimated the percentage of bivalve burrowing (using a 5-level scale: 0, 25, 50, 75 and 100%) by comparing the length of the part of the shell below the substratum surface with the part of the shell protruding above the substratum (according to Poznańska-Kakareko et al. 2021). Mean burrowing level was calculated according to the following formula:

$$MB = \sum_{i=1}^{4} [25\% * it_i] / \sum_{i=0}^{4} t_i \quad (1)$$

where: i – burrowing level: 5 steps ranging from 0 (totally exposed on the surface) to 4 (fully burrowed; t_i – time spent by the mussel at burrowing level i.

Statistical analysis

Statistical analysis was carried out using SPSS 26.0 (IBM Inc.). We checked bivalve habitat preferences in Experiment 1 using χ^2 tests of goodness of fit to compare their distribution within a given pair of habitats to a random distribution (assuming equal numbers of individuals selecting each habitat). Because of the high departures of the mobility and burrowing data in Experiment 2 from normality and homoscedasticity assumptions (tested with Shapiro-Wilk and Levene tests, respectively), we compared bivalve behaviour (each species separately) on each substratum contaminated with *S. woodiana* to their behaviour on pure sand using non-parametric Mann-Whitney U tests with a sequential Bonferroni correction for multiple comparisons.

Results

Experiment 1: Habitat selection

Both native species avoided small shells of *S. woodiana* (both burrowed and on the surface) at a density of 200 ind. m^{-2} (Fig. 1a, b, Table 2) and burrowed large shells at a density of 133 ind. m^{-2} (Fig. 1d, Table 2). Large shells on the surface were avoided at a density of 200 (*A. cygnea*) or 133 (*U. tumidus*) ind. m^{-2} (Fig. 1c, Table 2).

Burrowed shells were avoided in favour of shells of the same size and density (200 ind. m^{-2} of small shells) located on the substratum surface (Fig. 1e, Table 2). Large burrowed shells were avoided by *U. tumidus* in favour of small burrowed shells of the same total volume (Fig. 1f, Table 2).

The bivalves did not discriminate between living *S. woodiana* and pure sand (Fig. 1g, Table 2). *Unio tumidus* moved to the habitat formed by living *S. woodiana* avoiding large shells burrowed in the substratum, whereas *A. cygnea* did not discriminate significantly between these habitats (Fig. 1g, Table 2).

Unio tumidus showed a tendency to avoid shells of the native species, though it was non-significant (Fig. 2a, Table 2). Moreover, *U. tumidus* did not discriminate between shells of the native species and those of *S. woodiana*.

Sinanodonta woodiana avoided conspecific shells and did not discriminate between them and shells of the native unionids (Fig. 2b, Table 2).

Experiment 2: Bivalve mobility

Time from the introduction to the first movement of *A. cygnea* was not affected by the presence of shells and living individuals of *S. woodiana* (Fig. 3a, Table 3). *Unio tumidus* delayed the start of their activity in the presence of living *S. woodiana* (Fig. 3a, Table 3). Shells and living individuals of *S. woodiana* did not affect significantly the duration and distance of locomotion of *A. cygnea* (Fig. 3b, c, Table 3).

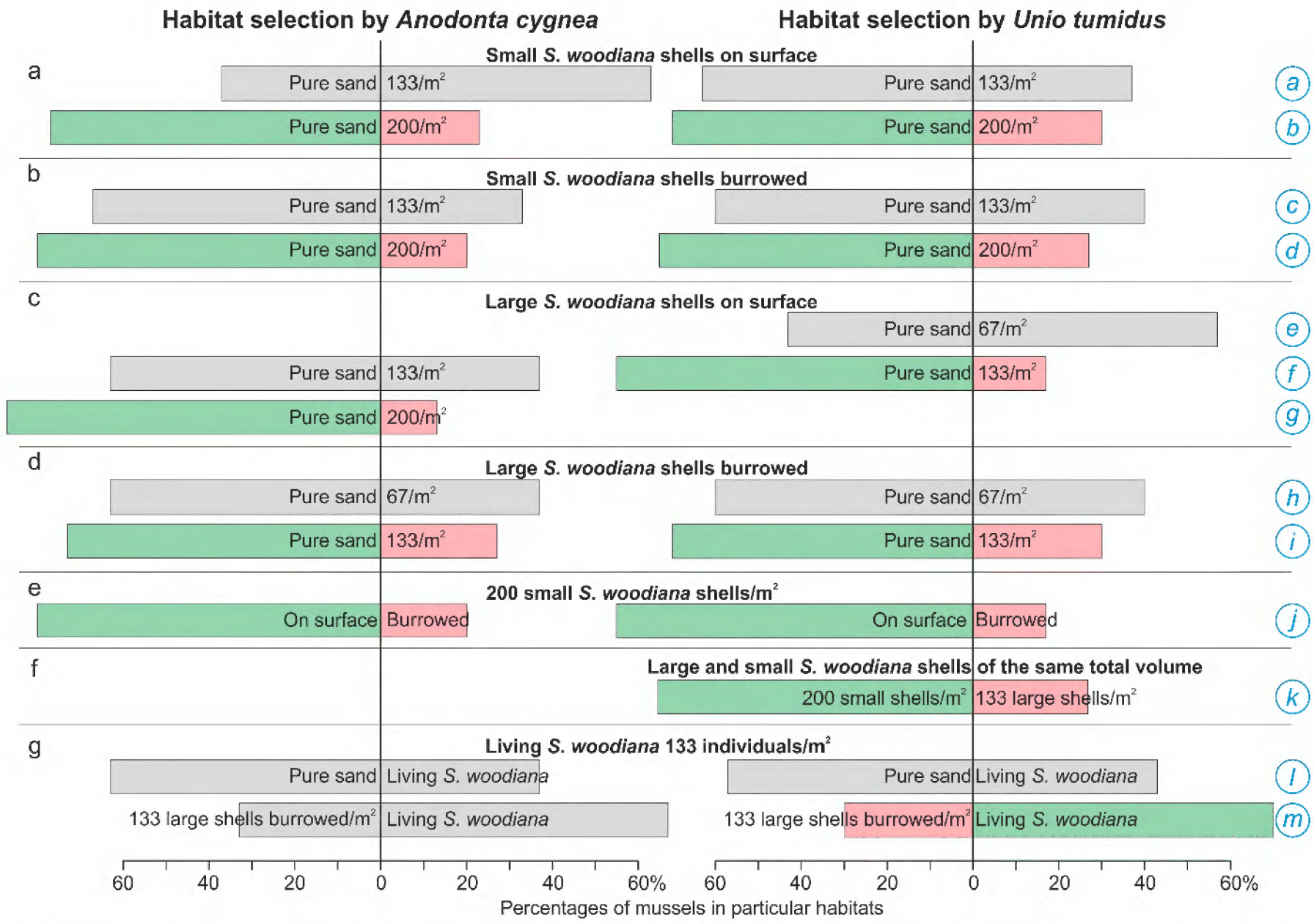

Figure 1. Habitat selection by *A. cygnea* and *U. tumidus* in the presence of substrata contaminated by *S. woodiana* in Experiment 1. Selected and avoided substrata are marked in green and red, respectively. The grey colour indicates non-significant differences. Blue letters in circles on the right refer to specific statistical tests presented in Table 2.

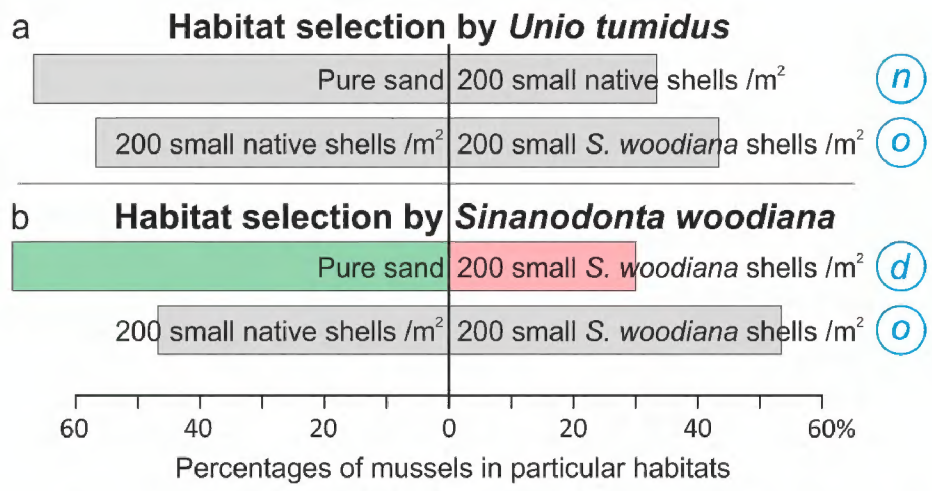
a
Habitat selection by Unio tumidus
Pure sand 200 small native shells /m²
200 small native shells /m² 200 small S. woodiana shells /m²
b
Habitat selection by Sinanodonta woodiana
Pure sand 200 small S. woodiana shells /m²
200 small native shells /m² 200 small S. woodiana shells /m²
60 40 20 0 20 40 60%
Percentages of mussels in particular habitats

Figure 2. Habitat selection of *U. tumidus* and *S. woodiana* in the presence of burrowed shells of native and invasive bivalves in Experiment 1. Selected and avoided substrata are marked in green and red, respectively. The grey colour indicates non-significant differences. Blue letters in circles on the right refer to specific statistical tests presented in Table 2.

However, in the presence of all forms of *S. woodiana* contamination, distances travelled by *A. cygnea* were slightly shorter and movement duration longer, resulting in a significantly slower crawling speed compared to that observed in the pure sand (Fig. 3d, Table 3).

Unio tumidus increased duration and distance of their locomotion in the presence of living *S. woodiana* or its shells on the surface (Fig. 3b, c, Table 3). In the pure sand and with burrowed shells, *U. tumidus* usually did not move horizontally at all, but burrowed immediately. Due to the total lack of locomotion of *U. tumidus* in the pure sand, it was not possible to calculate their speed on this substratum.

Table 2. Statistical analysis of habitat selection by *A. cygnea*, *U. tumidus* and *S. woodiana* in Experiment 1 (χ^2 tests of goodness of fit comparing bivalve distribution within a given pair of habitats to the random distribution assuming no selection). Statistically significant differences are indicated by bold font and asterisks. χ^2 – test statistic, P – statistical significance.

	Substrata			*Anodonta cygnea*		*Unio tumidus*		*Sinanodonta woodiana*	
				χ^2	*P*	χ^2	*P*	χ^2	*P*
a	control (pure sand)	vs.	133 small SW shells m^{-2} on surface	2.13	0.144	2.13	0.144	–	–
b	control (pure sand)	vs.	200 small SW shells m^{-2} on surface	8.53	**0.003***	4.80	**0.028***	–	–
c	control (pure sand)	vs.	133 small burrowed SW shells m^{-2}	6.53	0.068	4.80	0.273	–	–
d	control (pure sand)	vs.	200 small burrowed SW shells m^{-2}	10.80	**0.001***	6.53	**0.011***	4.80	**0.028***
e	control (pure sand)	vs.	67 large SW shells m^{-2} on surface	–	–	0.53	0.465	–	–
f	control (pure sand)	vs.	133 large SW shells m^{-2} on surface	2.13	0.144	13.33	**<0.001***	–	–
g	control (pure sand)	vs.	200 large SW shells m^{-2} on surface	16.13	**<0.001***	–	–	–	–
h	control (pure sand)	vs.	67 large burrowed SW shells m^{-2}	2.13	0.144	1.20	0.273	–	–
i	control (pure sand)	vs.	133 large burrowed SW shells m^{-2}	6.53	**0.011***	4.80	**0.028***	–	–
j	200 small SW shells m^{-2} on surface	vs.	200 small burrowed SW shells m^{-2}	10.80	**0.001***	13.33	**0.000***	–	–
k	200 small burrowed SW shells m^{-2}	vs.	133 large burrowed SW shells m^{-2}	–	–	6.53	**0.011***	–	–
l	control (pure sand)	vs.	133 living SW m^{-2}	2.13	0.144	0.53	0.465	–	–
m	133 large burrowed SW shells m^{-2}	vs.	133 living SW m^{-2}	3.33	0.068	4.80	**0.028***	–	–
n	control (pure sand)	vs.	200 small burrowed native shells m^{-2}	–	–	3.33	0.068	–	–
o	200 small burrowed native shells m^{-2}	vs.	200 small burrowed SW shells m^{-2}	–	–	0.53	0.465	0.13	0.715

Experiment 2: Bivalve burrowing

Anodonta cygnea spent a shorter time on burrowing in the substratum containing burrowed shells compared to the control sand (Fig. 4a, Table 3). There was no effect of *S. woodiana* habitats on the duration of burrowing activity of *U. tumidus*.

The mean burrowing level of *A. cygnea* was reduced in the presence of burrowed shells and living *S. woodiana* (Fig. 4b, Suppl. material 1: fig. S2, Table 3). *Unio tumidus* responded to all types of *S. woodiana* habitats by reducing its burrowing level.

Discussion

In accordance with our first hypothesis, we reported avoidance of *S. woodiana* shells by native unionids. On the other hand, living individuals of the invasive species were not avoided even at a density twice as high (133 ind. m^{-2}) as the maximum densities observed so far in the field (Kraszewski and Zdanowski 2007). However, living *S. woodiana* did influence unionid behaviour: their presence delayed initiation of activity and increased horizontal locomotion of *U. tumidus*, reduced locomotion speed in *A. cygnea*, and reduced burrowing of both species. An increased locomotion was also exhibited by *U. delphinus* Spengler, 1783 in the presence of the invasive clam *Corbicula* sp. (Ferreira-Rodríguez et al. 2018). It appears that the presence of shells induced displacement of native unionids, whereas living *S. woodiana* impaired the habitat quality for the natives, which tried to counteract by changing their activity. The increase in activity may be induced by searching for a habitat free of competitors, but its side effect may be the displacement of native bivalves to suboptimal environments, where they will be exposed to increased water flow or predatory pressure (Block et al. 2013). Another consequence of increased locomotion can be the depletion of energetic resources. As

Table 3. Statistical analysis of locomotion and burrowing of *A. cygnea* and *U. tumidus* in Experiment 2. Bivalve behaviour in the presence of *S. woodiana* shells (200 ind. m^{-2}, on the surface or burrowed) and living *S. woodiana* (133 ind. m^{-2}) was compared to the behaviour of individuals exposed on the control pure sand with pairwise Mann-Whitney U tests. Statistically significant differences are marked with asterisks and those that are still significant with the sequential Bonferroni correction are marked in bold font. Z – test statistic, P – statistical significance.

	Variable	Substrata			*Anodonta cygnea*		*Unio tumidus*	
					z	*P*	z	*P*
a	Movement initiation time	control (pure sand)	vs.	shells on surface	2.30	0.022*	0.62	0.534
				shells burrowed	1.52	0.129	0.77	0.443
				living individuals	0.23	0.818	3.59	**<0.001***
b	Locomotion duration	control (pure sand)	vs.	shells on surface	1.33	0.184	2.67	**0.008***
				shells burrowed	1.16	0.245	1.79	0.073
				living individuals	1.05	0.293	2.40	**0.017***
c	Locomotion distance	control (pure sand)	vs.	shells on surface	1.00	0.319	2.67	**0.008***
				shells burrowed	1.22	0.223	1.79	0.073
				living individuals	0.78	0.438	2.40	**0.017***
d	Locomotion speed	control (pure sand)	vs.	shells on surface	2.49	**0.013***	–	–
				shells burrowed	2.44	**0.015***	–	–
				living individuals	2.10	**0.035***	–	–
e	Duration of burrowing activity	control (pure sand)	vs.	shells on surface	0.73	0.467	1.81	0.071
				shells burrowed	3.32	**0.001***	0.56	0.575
				living individuals	1.83	0.067	1.14	0.254
f	Mean burrowing level	control (pure sand)	vs.	shells on surface	1.06	0.290	2.64	**0.008***
				shells burrowed	3.11	**0.002***	2.61	**0.009***
				living individuals	2.63	**0.009***	4.54	**<0.001***

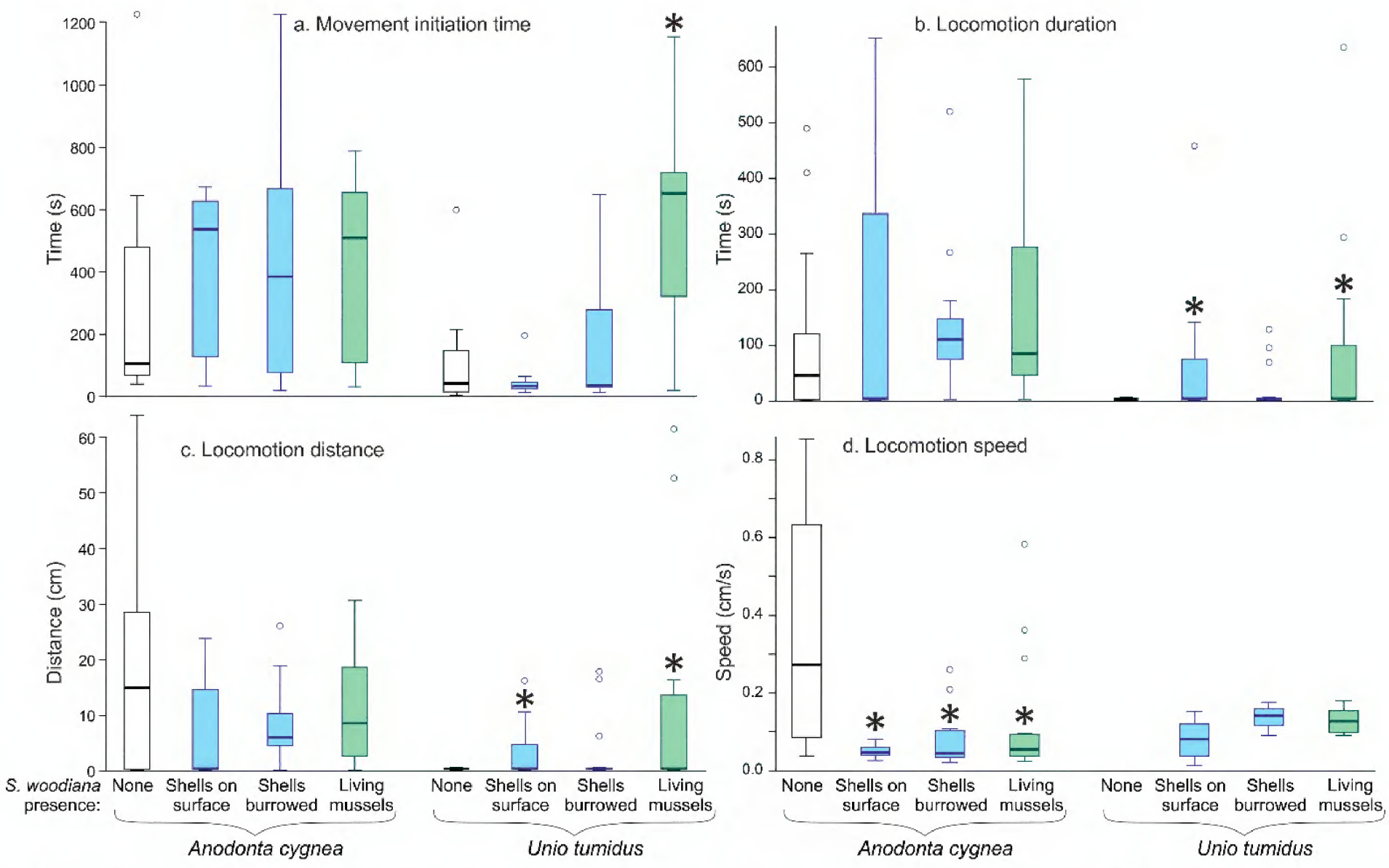

Figure 3. Mobility of *A. cygnea* and *U. tumidus* in Experiment 2: in pure sand (white bars), in the presence of *S. woodiana* shells (small shells, 200 ind. m^{-2}, blue bars) and in the presence of living *S. woodiana* (133 ind. m^{-2}, green bars) **a** movement initiation time **b** locomotion duration **c** locomotion distance and **d** locomotion speed. Asterisks indicate statistically significant differences in behaviour compared to that observed in the pure sand (see Table 3a-d for details of statistical test results). Boxplots present medians (horizontal lines), 1st and 3rd quartiles (boxes), 1.5*interquartile range (whiskers) and outliers (circles).

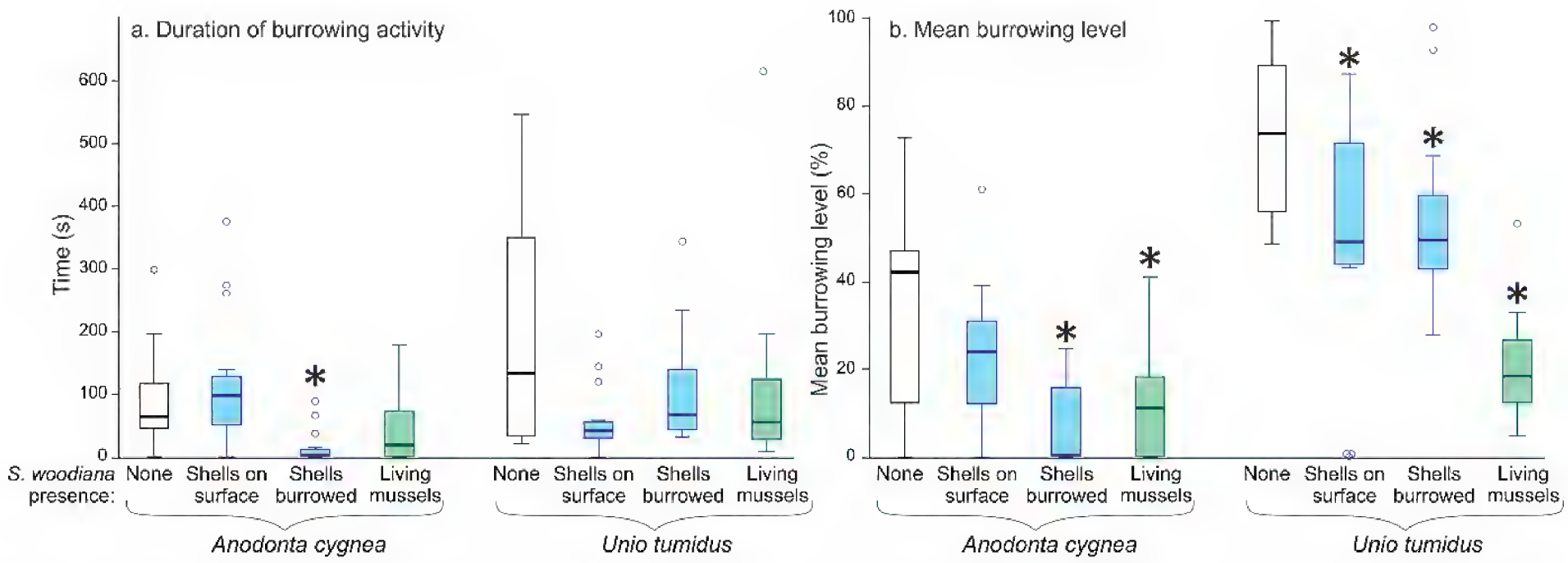

Figure 4. Burrowing of *A. cygnea* and *U. tumidus* in Experiment 2: in pure sand (white bars), in the presence of *S. woodiana* shells (small shells, 200 ind. m^{-2}, blue bars) and in the presence of living *S. woodiana* (133 ind. m^{-2}, green bars) **a** duration of burrowing activity **b** mean burrowing level (expressed as the percentage of bivalve length, see formula (1)). Asterisks indicate statistically significant differences in behaviour compared to that observed in the pure sand (see Table 3e-f for details of statistical test results). Boxplots present medians (horizontal lines), 1st and 3rd quartiles (boxes), 1.5*interquartile range (whiskers) and outliers (circles).

similar changes in behaviour took place in the presence of empty shells, they most likely resulted from mechanical properties of shells (acting as physical obstacles), rather than from infochemicals released to the water column by living *S. woodiana*.

As expected (third hypothesis), empty shells had a more aversive effect on native bivalves than living *S. woodiana*. The key result here is that the native bivalves avoided burrowed shells of *S. woodiana* to a greater extent than shells lying on the surface or living bivalves. The strongest effect of burrowed shells, immobilized in sediments, suggests that they are more difficult to push away by a moving mussel (compared to loose shells on the surface). Moreover, sharp shell edges, absent in living individuals, may irritate the foot of bivalves and discourage them from entering such a substratum. This was confirmed by the fact that *U. tumidus* did not increase their locomotion activity in the presence of burrowed shells, as they did among shells on the surface or with living *S. woodiana*. Thus, burrowed shells not only prevented mussels from entering the area, but also made it more difficult to leave a shell habitat when already present around the moving unionid. On the other hand, increased locomotion of *U. tumidus* among shells on the surface associated with their avoidance of such habitats indicates the active selection of shell-free habitats. The locomotion of *A. cygnea* in the presence of shells in or on the substratum resulted in similar distances as without shell beds, but at the cost of slower speed. This suggests a greater effort needed to obtain the same final effect, though, despite this, mussels continued to move in the presence of shells, also suggesting the active avoidance of shell beds by this species.

Avoidance of burrowed shells is related to their effect on the bivalve behaviour. We did note the negative effect of burrowed objects (empty shells and living *S. woodiana*) on the burrowing of both native species, especially *A. cygnea*. Moreover, *A. cygnea* spent less time on burrowing in shell beds, probably to avoid excessive energy expenditure. Restricted burrowing may be dangerous for unionid mussels, as being immersed in the substratum is their natural position, enabling their filtration, as well as reducing predation risk and the probability of dislodgement by water movements (Tallqvist 2001; Saloom and Duncan 2005). This indicates that the post-mortem effect of *S. woodiana* is two-faceted: it modifies the horizontal

and vertical mobility of bivalves, as predicted by our second hypothesis. This can substantially worsen the environmental conditions for native bivalves. We found that large shells are more aversive than small ones at the same volumetric quantities. This supports the idea that shells act as physical objects interfering with the movement of living mussels (a single large shell is more difficult to push away or bypass than a group of small ones). Thus, the impact of the invader is likely to be greater than that experienced by the native species before the invasion due to the much larger size of *S. woodiana* compared to native unionids, as predicted by our fourth hypothesis.

Native unionids can also create shell beds (Bódis et al. 2014a), which, as we have shown in our study, can exert a similar impact on living bivalves. However, their formation is slower than in the case of *S. woodiana*, which is capable of reaching high abundance and large body size in a relatively short time (Urbańska et al. 2019). Taking into account the thermophilicity of *S. woodiana* (Yermoshyna and Pavliuchenko 2021), it should be expected that, with progressing climate warming, mussels will reach larger sizes, higher density and biomass (Kraszewski and Zdanowski 2007; Spyra et al. 2012; Mehler et al. 2024) in temperate waters. This will increase their impact on local communities (Gutiérrez et al. 2003; Bellard et al. 2012) to an unprecedented level, not experienced so far by the native bivalves. It may be manifested by intensified competition for space, given that *S. woodiana* and the native unionids have similar substratum preferences (Poznańska-Kakareko et al. 2021), and by reduction in available area of optimum substratum due to the presence of extensive shell beds (Gutiérrez et al. 2003; Bódis et al. 2014a).

We have shown that *S. woodiana* also avoids habitats transformed by shells of its own species. This behaviour suggests that small scale spread of *S. woodiana* may be additionally stimulated by changes generated by this species in the environment, resulting in the occupation of a greater bottom area at an invaded site. Such a transformation of the environment by an ecosystem engineer associated with a negative feedback on its own living conditions is similar to the activity of cormorants (*Phalacrocorax* sp.), which pollute their surroundings (trees on which they nest in large numbers) with corrosive excrements and then move to new, not yet destroyed habitats (Ishida 1996).

Our results highlight that apart from competition for host-fish, food resources or living space, *S. woodiana* poses a further threat to native unionid bivalves by altering their horizontal and vertical movement behaviour. Whilst our tests were carried out in strictly controlled, specific conditions (i.e. stagnant water on sandy bottom), long-term negative impacts of living *S. woodiana* on native unionids can be expected to be even stronger than demonstrated in our study. We can expect that additional factors, such as water flow, different substratum or temperature would modify the relationships and behaviours observed in our study (Sullivan and Woolnough 2021). In our study, we only considered the physical effect of shells and living individuals of *S. woodiana*, showing the stronger impact of the former. In the environment, living *S. woodiana* may have additional adverse effects on native bivalves, such as competition for food or host fish, or reduction in water oxygenation. To fully understand the interactions between invasive and native Unionidae bivalves, would require to conduct longer tests, both in laboratory and in the field, examining additional potential mechanisms of impact.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

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Author contributions

KW: Conceptualisation, Methodology, Resources, Investigation, Formal analysis, Visualisation, Writing - Original draft, Writing - Review and Editing; DS: Methodology, Resources, Investigation, Writing - Review and Editing; JK: Conceptualization, Methodology, Validation, Formal analysis, Data Curation, Visualization, Writing - Review and Editing; TK: Resources, Investigation, Writing - Review and Editing; ŁJ: Resources, Investigation, Writing - Review and Editing; MPK: Conceptualization, Methodology, Validation, Visualization, Formal analysis, Supervision, Project administration, Writing - Review and Editing

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary material

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Explanation note: **table S1.** Full list of pairwise comparisons conducted to check unionid habitat selection in Experiment 1. **table S2.** Full list of treatments, response variables and statistical comparisons to check changes in unionid locomotion and burrowing in response to *S. woodiana* presence in Experiment 2. **fig. S1.** Photographs of the experimental setup. Experiment 1: Habitat selection by bivalves in various configurations: (A) pure sand (control) vs. 133 ind. m-2 of small *S. woodiana* shells on surface; (B) pure sand vs. 200 ind. m-2 of small shells on surface; (C) pure sand vs. 133 ind. m-2 of small burrowed shells; (D) pure sand vs. 200 ind. m-2 of small burrowed shells; (E) pure sand vs. 67 ind. m-2 of large shells on surface; (F) pure sand vs. 133 ind. m-2 of large shells on surface; (G) pure sand vs. 200 ind. m-2 of large shells on surface; (H) pure sand vs. 67 ind. m-2 of large burrowed shells; (I) pure sand vs. 133 ind. m-2 of large burrowed shells; (J) 200 ind. m-2 of small shells on surface vs. burrowed; (K) pure sand vs. 133 ind. m-2 of living *S. woodiana*; (L) 200 ind. m-2 of burrowed shells of native bivalves vs. shells of *S. woodiana*. **fig. S2**. Schemes of experimental sets concerning habitat selection (Experiment 1) and locomotion and burrowing (Experiment 2). **fig. S3.** Percentage of time spent in different levels of burrowing (Experiment 2) by *A. cygnea* and *U. tumidus* on different substrata. Error bars – standard deviation. **fig. S4.** Photo of immobilized *Sinanodonta woodiana*.

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